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(54) Title: GENERATION AND/OR REDUCTION OF NEW LUNG TISSUE IN AN AFFECTED LUNG

(57) Abstract: The present invention provides a means to influence the formation and/or reduction of new lung cells, by influencing a Wnt-pathway in an alveolar type II cell and/or alveolar type II tumor cell from said lung. Therefore, the invention provides a composition comprising a nucleic acid capable of binding at least a functional part of a nucleic acid encoding a protein which is involved in a Wnt-pathway in said cell, said binding influencing said Wnt-pathway. A composition of the invention may also comprise a protein capable of binding at least a functional part of a protein which is involved in a Wnt-pathway in said cell, or at least a functional part of a nucleic acid encoding a protein which is involved in a Wnt-pathway in said cell, said binding influencing said Wnt-pathway. A composition of the invention is suitable for the preparation of a medicament against emphysema, Respiratory Distress Syndrome and/or lung cancer.

Generation and/or reduction of new lung tissue in an affected lung

The invention relates to the field of medicine, more particularly to the treatment of lung diseases.

Worldwide, much investigation has been done on lung cells and diseases which affect lung cells, for instance emphysema and lung cancer. Until now, however, there is no efficient treatment of emphysema and lung cancer. In case of emphysema, patients suffer from shortness of breath, in first instance only on exertion, later on also at rest. This symptom may be accompanied by coughing, often with mucus expectorated. In later stages of the disease, heart failure occurs due to low oxygen levels in the blood circulation, often presenting as swollen ankles and liver enlargement. Pulmonary symptoms can be reduced by bronchodilator therapy and by use of courses of oral steroids. End-stage disease is treated with supplementation of oxygen by nasal canula. There is no treatment for the underlying cause of the disease. Consequently, most attention is being paid to decrease or even stop the process of dying of lung cells. Although some result has been obtained by the use of inhaled steroids, the lung damage continues which causes a progressive decrease in function (Pauwels et al., 1999; Burge, 2000). The problem is that even if said lung diseases can be counteracted, the lungs are already damaged by the disease. A solution to this problem would be the generation of new lung tissue. However, presently it is not possible to generate new lung tissue in a patient suffering from a lung disease.

In case of lung cancer, there are means of counteracting growth of the tumor. However, presently there is no medication which decreases the number of tumor cells in every patient. Decreasing the number of

tumor cells is highly favorable, because that would actually cure the disease. Until now, there is no general effective treatment for all kinds of lung cancer.

The present invention provides a new approach to counteract diseases which affect lung cells. In one embodiment the invention provides a means to counteract diseases which decrease the number of lung cells. The present invention does not only decrease the number of dying or abnormal cells. The invention discloses the uncommon and surprising approach to influence the number of viable lung cells in an affected lung. If said number is increased, the lung is able to at least partially recover from damage caused by a disease which was not efficiently, if at all, possible before the present invention.

The invention provides a way to influence the number of lung cells by influencing a Wnt-mediated signaling pathway (referred to in this disclosure as Wnt-pathway) in said cells. The Wnt gene family encodes developmentally important secreted factors, involved in cell growth, differentiation and organogenesis (Wodartz & Nusse, 1998). Wnt signaling events are initiated by receptor activation involving binding to the cysteine-rich domain (CRD) of frizzled 7-transmembrane receptor protein (Fz) (Bhanot et al., 1996). A classical Wnt signal suppresses the activity of glycogen synthase kinase 3 (GSK-3), leading to changes in phosphorylation and increased stability of the β-catenin protein in the cytoplasm (Hinck et al., 1994). β-catenin is essential for activating target genes in response to Wnt signaling (Miller & Moon, 1996; Willert & Nusse, 1998), since it complexes with HMG box transcription factors of the TCF/LEF family (Behrens et al., 1996; Molenaar et al., 1996; Huber et al., 1996). It has been shown that the presence of proteins that are able to bind Wnt proteins

through the CRD likely antagonize their actions. Amongst these are the so-called secreted Frizzled-related (sFRPs) proteins (Leyns et al., 1997; Wang et al., 1997) and Dickkopf proteins. Dickkopf proteins are potent Wnt antagonists (Glinka et al., 1998).

Components of the Wnt signaling pathway have been found to be present during organogenesis in the mouse (Roelink & Nusse, 1991; Buhler et al., 1993; Parr et al., 1993; Christianses et al., 1995; Wang & Shackleford, 1996; Cho & Dressler, 1998; Korinek et al., 1998; Leimester et al., 1998; Oosterwegel et al., 1993). Moreover, loss of function of Wnt and Wnt-related genes leads to abnormal development in the mouse (McMahon & Bradley, 1990; Monkley et al., 1993; Takada et al., 1994; Stark et al., 1994; Galceran et al., 1999; Liu et al., 1999; Yamaguchi et al., 1999; Brisken et al., 2000; Lee et al., 2000). Several Wnts and components of the Wnt pathway are expressed in the murine lung in the course of its development (Gavin et al., 1990; Levay-young et al., 1996; Katoh et al., 1996; Lako et al., 1998; Zakin et al., 1998; Imai & D'Armiento, 1999). This shows that Wnt signaling is important for normal lung morphogenesis.

In one aspect the present invention provides a composition capable of influencing the proliferation and/or differentiation behavior of an alveolar type II cell and/or an alveolar type II tumor cell from a lung, comprising a nucleic acid capable of binding at least a functional part of a nucleic acid encoding a protein which is involved in a Wnt-pathway in said cell, said binding influencing said Wnt-pathway.

Alveolar type II cells arise at a specific stage of lung development as has been reported for the mouse (Ten Have-Opbroek, 1975; 1979; 1981; 1991) and other species

including humans (Otto-Verberne and Ten Have-Opbroek, 1987; Otto-Verberne et al., 1988; Ten Have-Opbroek and Plopper, 1992). In the mouse embryo, the lung primordium appears at about 9.5 days after conception (a.c.) (Ten Have-Opbroek, 1981; 1991). It develops into the prospective trachea and two lung buds. The latter give rise to the primordial system of the right and left lungs, which is composed of primordial tubules lined by undifferentiated pseudostratified columnar epithelium. From 14.2 days a.c. onward, the primordial system differentiates into the prospective bronchial system and the prospective alveolar system (unit: pulmonary acinus). The pulmonary acinus consists of tubules called acinar tubules (Ten Have-Opbroek, 1979). While the epithelium of the bronchial tubules is columnar, the epithelial lining of the acinar tubules is low-columnar or cuboid and composed of prospective alveolar type II cells (Ten Have-Opbroek, 1979; Ten Have-Opbroek et al., 1988). This is the so-called pseudoglandular period of lung development, which lasts until day 16.6 a.c. In later stages of lung development (i.e canalicular, terminal sac and alveolar periods), a further development of the bronchial and alveolar systems takes place, and the acinar tubules start to transform into derivative structures with a duct-, sac- or pouch-like shape. The epithelial lining of these structures now also contains flatter cells, which are prospective alveolar type I cells (Ten Have-Opbroek et al., 1990). Alveolar type II cells play an important role in the formation of the pulmonary acinus, because they are the only dividing alveolar epithelial cells and the stem cells for the alveolar type I cells. Alveolar type II cells are (one of the) predominant stem cells in the development of the two major subsets of non-small cell lung cancer, namely adenocarcinomas and squamous cell carcinomas (Ten Have-Opbroek et al., 1990; 1993; 1994; 1996; 1997; 2000).

Proliferation of an alveolar type II cell is defined as dividing of said cell, forming more cells.

Differentiation of an alveolar type II cell is defined as changing of said cell into a mature alveolar type II cell, or into another kind of cell, said other kind of cell having for instance a different shape and/or function. One example is the change of an alveolar type II cell into an alveolar type I cell.

A composition of the invention may comprise a nucleic acid capable of binding at least a functional part of a nucleic acid encoding a protein which is involved in a Wnt-pathway in said cell. Said binding influences expression of said protein. This way, said binding influences said Wnt-pathway.

Alternatively, a composition of the invention may comprise a protein which is capable of binding at least a functional part of a protein which is involved in a Wnt-pathway. Binding of a protein of the invention to said protein which is involved in a Wnt-pathway, changes the properties of said protein which is involved in a Wnt-pathway. This way, said Wnt-pathway is influenced.

A composition of the invention may also comprise a protein which is capable of binding at least a functional part of a nucleic acid encoding a protein which is involved in a Wnt-pathway in said cell. Binding of a protein of the invention to said functional part of a nucleic acid influences expression of said protein which is involved in a Wnt-pathway in said cell. Said binding, for instance, inhibits expression of said protein. This influences the Wnt-pathway.

Thus, another embodiment of the invention provides a composition capable of influencing the proliferation and/or differentiation behavior of an alveolar type II cell and/or an alveolar type II tumor cell from a lung,

comprising a protein capable of binding at least a functional part of a protein which is involved in a Wnt-pathway in said cell, or at least a functional part of a nucleic acid encoding a protein which is involved in a Wnt-pathway in said cell, said binding influencing said Wnt-pathway.

A functional part of a nucleic acid is defined as a part which is essential for expression of a protein. Said functional part may for instance encode a functional part, derivative, and/or analogue of said protein.

A functional part of a protein is defined as a part which has the same kind of properties as said protein in kind, not necessarily in amount.

A functional derivative of a protein is defined as a protein which has been altered such that the properties of said derivative are essentially the same in kind, not necessarily in amount. A derivative can be provided in many ways, for instance through conservative amino acid substitution.

A person skilled in the art is well able to generate analogous compounds of a protein. This can for instance be done through screening of a peptide library. Such an analogue has essentially the same properties of said protein in kind, not necessarily in amount.

A composition of the invention may be used to generate more lung cells. This is for instance desirable if lung tissue has been damaged by a disease like emphysema. For more lung cells to be generated, a Wnt pathway may be upregulated. Thus in one aspect the invention provides a composition according to the invention, wherein said Wnt-pathway is upregulated.

In other cases, however, it may be desirable to stop proliferation and/or differentiation of lung cells. This

is for instance true if an individual suffers from lung cancer. It has been found that several components of Wnt signaling are implicated in the genesis of human cancer (Morin et al., 1997, Rubinfeld et al., 1997) including lung cancer (Winn et al., 2000). Therefore, in another aspect, the present invention discloses a means of decreasing the amount of lung tumor cells by downregulating a Wnt-pathway in said tumor cells.

A composition of the invention is capable of influencing the proliferation and/or differentiation behavior of an alveolar type II cell and/or alveolar type II tumor cell. Said cells may be located inside a body of a human or animal. However, other locations (*in vitro*) are possible. So in one aspect the invention provides a composition according to the invention, wherein said cell is located inside a body of a human or animal.

Proteins which are involved in a Wnt-pathway in a lung cell are for instance secreted Frizzled-related proteins (sFRPs) and Dickkopf proteins (Dkks). Said proteins counteract a Wnt-pathway, by binding to certain Wnt- or Wnt-related proteins and antagonizing their actions. So, in another aspect the invention provides a composition according to the invention, which is at least in part capable of inhibiting expression of at least one secreted Frizzled-related protein and/or Dickkopf protein. If said secreted Frizzled-related protein and/or Dickkopf protein is less expressed, less secreted Frizzled-related protein and/or Dickkopf protein will be present to counteract a Wnt-pathway.

Expression of a secreted Frizzled-related protein and/or Dickkopf protein may be inhibited by a nucleic acid which is capable of binding to at least a functional part of DNA and/or RNA encoding at least part of said

secreted Frizzled-related protein and/or Dickkopf protein. Said nucleic acid may be an antisense strand. If said DNA and/or RNA encoding at least part of secreted Frizzled-related protein and/or Dickkopf protein is bound by an antisense strand, expression of secreted Frizzled-related protein and/or Dickkopf protein is, at least in part, inhibited. Thus in one aspect the invention provides a compound according to the invention, which at least comprises one antisense strand of at least a functional part of DNA and/or RNA encoding at least part of secreted Frizzled-related protein and/or Dickkopf protein.

Alternatively, a Wnt-pathway may be influenced by influencing a Wnt-pathway inhibiting property of a secreted Frizzled-related protein and/or Dickkopf protein. Expression of secreted Frizzled-related protein and/or Dickkopf protein may remain the same in this case. In this case, the same amount of secreted Frizzled-related protein and/or Dickkopf protein may be present, but the Wnt-pathway inhibiting property of said protein has changed. Thus, in one aspect, the invention provides a compound according to the invention, which is capable of at least in part counteracting a Wnt-pathway inhibiting property of at least one secreted Frizzled-related protein and/or Dickkopf protein.

A Wnt-pathway inhibiting property of a secreted Frizzled-related protein and/or Dickkopf protein can be changed by binding of a compound to said secreted Frizzled-related protein and/or Dickkopf protein. Binding of a compound to said protein can for instance alter the conformation of said protein. A person skilled in the art can think of many other ways how binding of a compound to a protein can change its properties. Thus, another aspect of the invention discloses a compound according to the

invention, which is capable of binding to at least one secreted Frizzled-related protein and/or Dickkopf protein. Said binding compound may be an antibody. So in yet another aspect the invention provides a compound according to the invention, which comprises an antibody comprising a binding specificity against a secreted Frizzled-related protein and/or Dickkopf protein, or a functional part, derivative and/or analogue of said antibody. A functional part, derivative and/or analogue is defined herein as disclosed above.

We have demonstrated that expression of secreted Frizzled-related protein-1 (sFRP-1), sFRP-2, sFRP-3 and sFRP-4, and expression of Dickkopf protein Dkk1, Dkk2 and Dkk 3, in mouse embryos occurred during lung development (example 1). This suggests that at least these sFRP's and Dickkopf proteins are important for the proliferation and/or differentiation process of lung cells. Thus in one aspect the invention discloses a compound according to the invention, wherein said Frizzled-related protein is sFRP-1, sFRP-2, sFRP-3, and/or sFRP-4. The invention also discloses a compound according to the invention, wherein said Dickkopf protein is Dkk1, Dkk2 and/or Dkk3.

We have demonstrated that transcription factors of the TCF/LEF family are also involved in lung development in a mouse (example 1). Therefore, to influence proliferation and/or differentiation of a lung cell, one embodiment of the invention provides a compound according to the invention, which is capable of activating expression of at least one transcription factor of the TCF/LEF family. Said compound may for instance be an enhancer of transcription of a gene encoding said member of the TCF/LEF family. Alternatively, said compound may be a nucleic acid encoding said member of the TCF/LEF family. If said nucleic acid is administered to a cell,

expression of said member of the TCF/LEF family is increased. So one embodiment of the invention discloses a compound according to the invention, which at least comprises one nucleic acid encoding a transcription factor of the TCF/LEF family or a functional part, derivative and/or analogue thereof.

We have shown that at least transcription factors TCF-1, TCF-3, TCF-4 and/or Lef-1 are involved in lung development (table 1). Thus, one embodiment of the invention provides a compound according to the invention, wherein said transcription factor of the TCF/LEF family is TCF-1, TCF-3, TCF-4 and/or LEF-1.

Forming of new alveolar tissue in patients can be stimulated by (re)activation of formation of alveolar buds. This is an embryologic mechanism that is still active in the adult situation but at a much lower level (i.e. local concentrations of alveolar type II cells in connection with alveolar epithelial cell renewal). Formation of alveolar buds is based on active proliferation of alveolar type II cells. Formed alveolar buds proliferate into surrounding, eventually new induced, tissue. As a compound of the invention is capable of influencing said proliferation of alveolar type II cells, one embodiment of the invention provides a compound according to the invention, which is capable of inducing the formation of an alveolar bud.

Another important function of alveolar type II cells is synthesis and secretion of surfactant. Said surfactant regulates the surface tension in the alveoli. So a compound of the invention is also useful for individuals suffering from surfactant deficiency. Said individuals may suffer from Respiratory Distress Syndrome. Therefore, one embodiment of the invention provides a compound according to the invention, which is capable of inducing synthesis and/or secretion of surfactant by a lung cell.

Another embodiment of the present invention provides an isolated cell, comprising a compound according to the invention. Said compound may comprise a nucleic acid capable of binding at least a functional part of a nucleic acid encoding a protein which is involved in a Wnt-pathway in said cell. To provide a cell with said nucleic acid, said nucleic acid may be inserted into a vector. Thus, one embodiment of the invention provides a vector comprising a nucleic acid capable of binding at least a functional part of a nucleic acid encoding a protein which is involved in a Wnt-pathway in a cell, said binding influencing said Wnt-pathway.

A vector of the invention may also comprise a nucleic acid encoding a protein capable of binding at least a functional part of a protein which is involved in a Wnt-pathway in a cell, or at least a functional part of a nucleic acid encoding a protein which is involved in a Wnt-pathway in a cell, said binding influencing said Wnt-pathway.

A compound of the invention is particularly suited for the preparation of a medicament, especially for lung diseases. So in one aspect the invention provides a use of a compound according to the invention for the preparation of a medicament. Lung diseases which can be, at least in part, counteracted by a compound of the invention comprise emphysema, Respiratory Distress Syndrome, and lung cancer.

So in one aspect, the invention provides a use of a compound according to the invention for the preparation of a medicament for emphysema.

In another aspect, the invention provides a use of a compound according to the invention for the preparation of a medicament for Respiratory Distress Syndrome.

In yet another aspect, the invention provides a use of a compound according to the invention for the preparation of a medicament for lung cancer.

As a compound of the invention is capable of inducing the formation of an alveolar bud, yet another embodiment of the invention provides a method for inducing the formation of an alveolar bud, comprising administering a compound according to the invention to an alveolar type II cell.

Yet another embodiment provides a method for inducing synthesis and/or secretion of surfactant by a cell, comprising administering a compound according to the invention to said cell. Said cell may be an alveolar type II cell.

As a compound of the invention is, at least in part, capable of counteracting lung diseases like emphysema, Respiratory Distress Syndrome, and lung cancer, the invention provides in one aspect a method for, at least in part, treatment of emphysema, comprising administering a compound according to the invention to an individual.

In another aspect, the invention provides a method for, at least in part, treatment of Respiratory Distress Syndrome, comprising administering a compound according to the invention to an individual.

In yet another aspect, the invention provides a method for, at least in part, treatment of lung cancer, comprising administering a compound according to the invention to an individual.

In lung cancer, expression of components of the Wnt-pathway may be up- or down-regulated in the epithelial, mesenchymal or other cells causing enhanced proliferation of said cells. If a component of the Wnt-pathway is down-regulated (e.g. Wnt7a, Calvo et al., 2000), up-regulation of said component provides a means to slow down

proliferation of said cells. This can be achieved by replacement of said component, e.g. by administration of cells manipulated to express said Wnt component (e.g. Wnt 7a). However, components of a Wnt-pathway may also be up-regulated in lung cancer cells as is the case in e.g. colon cancer cells (Bienz & Clevers, 2000). Inhibition of the activity of said components can be used to reduce proliferation of the relevant cells. This may be achieved by antisense techniques as described before, e.g. by local administration in the airways of antisense oligos for beta-catenin, or another component of the Wnt-pathway that is up-regulated.

Thus, for treatment of lung cancer, expression of a component of the Wnt-pathway may have to be either up- or down-regulated, depending on the particular component.

The following, non-limiting, examples are meant to illustrate the invention. A person skilled in the art is capable to perform alternative experiments which are still in the scope of the present invention.

EXAMPLES

Example 1. Expression of Wnt-pathway components in alveolar epithelium and/or surrounding mesenchyme during murine lung development

Animals

In this study, an inbred Swiss-type mouse strain with a gestation time of about 19 days after conception (a.c.) was used. The embryos were obtained from female mice aged about 3 months and weighing 30-40 g. They carried 5 to 15 embryos, whose weight was used as a parameter of the developmental stage since it is a more sensitive indicator than the age in days a.c. A growth curve based on the relationship between weights and ages allowed us to determine what we call the "developmental age" of the mouse embryo (Goedbloed, 1976; Ten Have-Opbroek et al., 1988), which is indicated in the text for all embryos used.

Processing of the tissue

The pregnant mice were killed by cervical dislocation. The embryos were removed from the uterus and weighed to determine the developmental age. Then the lungs were removed from the mother and the embryos by thoracotomy, divided in two portions (the left lung consisting of one large lobe, and the right lung composed of four lobes) and fixed by immersion in 4% paraformaldehyde overnight at room temperature (rt).

Whole mount in situ hybridisation (ISH) probes

Both antisense and sense digoxigenin-labeled RNA probes were generated from LEF-1, TCF-1, TCF-3 and TCF-4 cDNAs, and from sFRP-1, sFRP-2, sFRP-3 and sFRP-4 cDNAs.

Whole mount ISH

After washing for 5 min in PBT (PBS containing 0.1% Tween-20), the specimens were dehydrated through a graded methanol series (25%, 50% and 75% in PBT for 5 min each, and 100% 2x for 5 min) and stored in methanol 100% at -20°C until use.

Whole mount ISH was performed essentially as described (Wilkinson and Nieto 1993, Wilkinson, 1995; Nieto et al., 1996) with minor modifications. Afterwards, the whole mount ISH samples were sectioned and mounted on slides to study the cellular localization of the mRNA signal.

Immunohistochemistry

Immunohistochemical staining was performed using the avidin-biotin complex (ABC) method with peroxidase labeling and 3-3'diaminobenzidine (DAB) as the chromogen (VECTOR; Burlingame, CA, USA). Briefly, the procedure involves the following steps: 1) hydration of the paraffin sections through xylene and a graded ethanol series (100-70%, each step lasting 30 min) and quenching of the endogenous peroxidase activity with 100% methanol containing 0.4% hydrogen peroxide (H_2O_2) for 20 min at rt; 2) 3 times rinsing in Tris Maleate buffer (TMB, pH 7.6) for 1 min at rt and incubation with 10% normal horse serum for 1 h at rt; 3) incubation with the primary antibody (anti β-catenin, anti LEF-1/TCFs and anti sFRPs; all diluted in PBS, pH 7.6) overnight at 4°C and rinsing in TMB; 4) incubation with a 1:400 dilution of biotinylated swine anti-rabbit IgG (DAKO, Denmark) or biotinylated horse anti-mouse IgG for 60 min at rt and rinsing in TMB; 5) incubation with ABC for 30 min at rt and rinsing in TMB; and 6) incubation with TMB containing 0.04% DAB and 0.006% H_2O_2 for 10 min at rt. Finally, the sections were washed in TMB for 1 min and in tap water

for 10 min, then counterstained with hematoxylin for 5 sec, rinsed in tap water for 10 min, dehydrated through a graded ethanol series (70-100%) and xylene and mounted with xylene-soluble mounting medium *Depex* (H.D. SUPPLIES, England).

Immunohistochemical controls were performed on the serial mouse fetal lung sections using normal rabbit or mouse IgG or serum as the primary antibody, or omission of one of the incubation steps.

RT-PCR analysis

Total RNA was isolated from lungs dissected from embryos and fetuses of different developmental stages (E 12-E18), neonates, 1 and 3 week olds and adults, using TriPure Isolation Reagent (Boehringer-Mannheim). RNA was quantified spectrophotometrically. cDNA was synthesized using random hexamers (Gibco BRL) and Superscript II Reverse Transcriptase (Gibco BRL). RT-PCR was performed with the following conditions: 100 μM random hexamers (Gibco BRL), 1 to 5 μg total RNA, 5 x First-Strand Buffer (Gibco BRL), 0.1 M DTT (Gibco BRL), 25 mM dNTPs (Gibco BRL), 40 units RNase OUT and 200 units of Superscript II RT (Gibco BRL) in 20 μl total volume. Reverse transcription reactions were performed in a Peltier Thermal Cycler PTC-200 (MJ Research). Reactions were incubated at 25 °C for 10 min to allow the hexamers to anneal followed by 50 min 42 °C for reverse transcription. PCR was conducted using 5 μl cDNA. PCR conditions were as follows: 10x Tfl buffer, 25 mM dNTP, 3 μM primer and 0.4 units Tfl DNA polymerase (Promega) in a total volume of 50 μl. PCR was performed at 1 cycle of 94 °C for 1 min, followed by temperature cycles varying from 20 to 35 times: 92 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. This was followed by a final 10 min extension at 72 °C. 4 μl of each reaction was analysed on a 1.5 %

agarose gel and visualized by ethidium bromide under UV light. Gels were photographed using APPLIGENE INC. imager Software version 2.0.

Example 2. *Activation of alveolar type II cells in a murine lung by influencing a Wnt-pathway*

To provide proof of evidence, a lung organoid culture (obtained from a mouse) is used. Generation of new alveolar tissue in patients (see p. 9) can be stimulated by activation or re-activation of alveolar bud formation. Alveolar bud formation is a general growth principle in both the fetal and the adult mammalian lung. As a proof of evidence, it is therefore shown that manipulation of expression and/or function of selected molecules, involved in a Wnt-pathway, stimulates the process of alveolar bud formation. Activation of alveolar type II cells by influencing a Wnt-pathway is for instance demonstrated using anti-sense oligonucleotides. These oligonucleotides may be directed against, e.g., sFRPs and/or DKKs. One subject of investigation is the means of administration of compositions capable of influencing a Wnt-pathway. The effect of administration is investigated using a biological *in vitro* and/or *in vivo* test-system, preferably the above-mentioned murine organoid lung culture.

The fetal murine organoid lung culture is generated using the protocol of prof. Zimmermann, Freie Universität, Berlin (Zimmermann, 1987; Zimmermann, 1989; Hundertmark et al., 1999). The presence of molecules involved in a Wnt-pathway in said murine lung culture is tested using molecular-biological methods as, e.g., *in situ* hybridisation and/or immunohistochemistry. Once said molecules involved in a Wnt-pathway are found, anti-sense

oligonucleotides against said molecules are generated. Modified stable anti-sense oligonucleotides are produced using existing protocols (Augustine et al., 1995; Dagle et al., 2000; Heasman et al., 2000) with adaptations and/or are obtained commercially. After that, the *in vitro* effect of said generated oligonucleotides is tested in the fetal murine lung culture. Said oligonucleotides are administered to the culture medium in different concentrations. The effective concentration of the administered oligonucleotides, capable of influencing formation of alveolar tissue, is determined experimentally using morphological and/or biochemical techniques. For instance, sections from treated alveolar tissues and untreated controls are investigated by histochemistry, immunohistochemistry and/or morphometry. Criteria are for instance the ratio between primordial lung cells and alveolar type II cells in the lung buds, and/or the increase of the number of alveolar type II cells, and/or proliferating alveolar type II cells, per cm basal membrane. Other criteria include the number of alveolar spaces, the size of the gas exchange surface, and the weight and/or volume of the lung (Otto-Verberne et al., 1991; Brandsma et al., 1994; Heemskerk-Gerritsen et al., 1996). Additional information on alveolar type II cell differentiation is obtained by electronmicroscopic research, by biochemical investigation, like for instance surfactant protein A (SP-A) detection in the culture medium and/or by detection of relevant RNAs using in situ hybridization (ISH) and polymerase chain reaction (PCR). Similar approaches are used for biological test-systems in neonatal and/or adult murine lungs.

Preferably, results concerning the formation of alveolar tissue are obtained using anti-sense oligonucleotides, or combinations of anti-sense oligonucleotides, which disturb the type II cell

equilibrium. More preferably, said oligonucleotides inhibit differentiation in favour of proliferation.

Organoid lung cultures

The murine organoid lung culture is generated using the protocol of prof. Zimmermann, Freie Universität, Berlin (Zimmermann, 1987; Zimmermann, 1989; Hundertmark et al., 1999). Briefly, the lungs are homogenized and the homogenates are cultured for 2 to 3 weeks.

Oligonucleotides

Modified stable anti-sense oligonucleotides are produced using existing protocols and/or obtained commercially. (Augustine et al., 1995; Dagle et al., 2000; Heasman et al., 2000).

Controls: As a control of said oligonucleotides sense and/or mismatch and/or scrambled control oligonucleotides are either produced or obtained commercially. For exploration purposes some of these oligos are provided with a fluorescein label.

Means of administration: The nucleotides are administered using, e.g., osmotic and/or scrape delivery and/or syringe loading and/or enzyme treatment and/or electroporation and/or by poly ethylenimines e.g. EPEI or ExGen 500.

Effective concentration: The effective concentration of the oligonucleotides is determined by the biological criteria mentioned above.

RT-PCR analysis

Total RNA was isolated from the murine organoid lung cultures using TriPure Isolation Reagent (Boehringer-Mannheim). RNA was quantified spectrophotometrically. cDNA was synthesized using random hexamers (Gibco BRL)

and Superscript II Reverse Transcriptase (Gibco BRL). RT-PCR was performed with the following conditions: 100 µM random hexamers (Gibco BRL), 1 to 5 µg total RNA, 5 x First-Strand Buffer (Gibco BRL), 0.1 M DTT (Gibco BRL), 25 mM dNTPs (Gibco BRL), 40 units RNase OUT and 200 units of Superscript II RT (Gibco BRL) in 20 µl total volume. Reverse transcription reactions were performed in a Peltier Thermal Cycler PTC-200 (MJ Research). Reactions were incubated at 25 °C for 10 min to allow the hexamers to anneal followed by 50 min 42 °C for reverse transcription. PCR was conducted using 5 µl cDNA. PCR conditions were as follows: 10x Tfl buffer, 25 mM dNTP, 3 µM primer and 0.4 units Tfl DNA polymerase (Promega) in a total volume of 50 µl. PCR was performed at 1 cycle of 94 °C for 1 min, followed by temperature cycles varying from 20 to 35 times: 92 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. This was followed by a final 10 min extension at 72 °C. 4 µl of each reaction was analysed on a 1.5 % agarose gel and visualized by ethidium bromide under UV light. Gels were photographed using APPLIGENE INC. imager Software version 2.0.

Example 3. Expression of Wnt signalling pathway components in human lung tissues

Human lung tissues

Normal control and diseased (notably emphysematous, cancerous) human lung tissue is obtained by surgery.

RT-PCR analysis

Total RNA was isolated from normal and emphysematous lung tissue from adult human lungs using TriPure Isolation Reagent (Boehringer-Mannheim). RNA was quantified spectrophotometrically and cDNA was

synthesized using random hexamers (Gibco BRL) and Superscript II Reverse Transcriptase (Gibco BRL). RT-PCR was performed with the following conditions: 100 µM random Hexamers (Gibco BRL), 1 to 5 µg total RNA, 5 x First-Strand Buffer (Gibco BRL), 0.1 M DTT (Gibco BRL), 25 mM dNTPs (Gibco BRL), 40 units RNase OUT and 200 units of Superscript II RT (Gibco BRL) in 20 µl total volume. Reverse transcription reactions were performed in a Peltier Thermal Cycler PTC-200 (MJ Research). Reactions were incubated at 25 °C for 10 min to allow the hexamers to anneal followed by 50 min 42 °C for reverse transcription. PCR was conducted using 5 µl cDNA. PCR conditions were as follows: 10x Tfl buffer, 25 mM dNTP, 3 µM primer and 0.4 units Tfl DNA polymerase (Promega) in a total volume of 50 µl. PCR was performed at 1 cycle of 94 °C for 1 min then a temperature cycle varies from 20 to 35 times: 92 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. This was followed by a final 10 min extension at 72 °C. 4 µl of each reaction was analysed on a 1.5 % agarose gel and visualized by etidium bromide under UV light. Gels were photographed using APPLIGENE INC. The imager Software version 2.0.

Results:

Example 1. *Expression of Wnt-pathway components in alveolar epithelium and/or surrounding mesenchyme during murine lung development*

1) Whole mount ISH data:

TCF-1 mRNA was clearly expressed around 11 days a.c., and it reached the maximum levels between 13 and 15 days a.c. Interestingly, TCF-1 mRNA expression remained slightly positive through 16, 17 and 18 days a.c., and also in the adult lung. mRNA coding for TCF-3 was found to be expressed as early as 10 days a.c. Its expression levels were high from 12 days a.c. till 16 days a.c., and began to decrease between 17 and 18 days a.c. Regarding TCF-4 mRNA expression, similar to those of TCF-3, it was present already at 10 days a.c. and achieved the highest levels around 12 days a.c. However, in contrast to the other transcription factors studied, at 13 days a.c. the TCF-4 mRNA expression declined and it was nearly negative at 14 days a.c. Finally, mRNA coding for LEF-1 was found positive at 11 days a.c. The signal was elevated during 12, 13, 14 and 15 days a.c. At 16 days a.c., LEF-1 mRNA expression decreased and was negative at 17 d.a.c.

2) Sections of the whole mount ISH samples:

The sectioning of the whole mount ISH samples showed the cellular localization of the mRNA expression for the TCFs/LEF-1 transcription factors. TCF-1 mRNA expression appeared to be located in the mesenchymal cells in close proximity to the alveolar epithelial cells, but also in the apical cytoplasmic areas of the epithelial cells lining the lung primordia and acinar tubules. For TCF-3, the mRNA expression was present mainly in the apical side of the alveolar epithelial cells, similar to the signal

corresponding to TCF-4 mRNA. Finally, LEF-1 mRNA expression was located just in the mesenchyme around the epithelial lining of the lung primordia and acinar tubules.

Protein expression of β-catenin, LEF1/TCFs and sFRPs during murine lung development.

At 13 days a.c., the protein expression corresponding to β-catenin was found to be present in the cell junctions of the prospective bronchial epithelium, while the alveolar epithelial cells lining the acinar tubules (prospective respiratory epithelium) showed β-catenin protein expression in the cytoplasm as well as in the nuclei. Later on during development (around 17 days a.c.), the differentiating alveolar type I cells were negative for the expression of this protein, while some alveolar type II cells were still positive.

LEF-1/TCFs protein cytoplasmic expression was present in the epithelial cells (prospective bronchial and respiratory epithelium and/or in the mesenchyme) at 13 days a.c. At 17 days a.c., some TCF expression was still present in the alveolar type II cells.

For sFRP-protein, a slight expression was located mainly in the cytoplasm of the epithelial cells lining both the prospective bronchial and respiratory epithelium, but also in the mesenchyme, at 13 days a.c. The alveolar type II cells together with the differentiating alveolar type I cells were found to be negative for sFRPs protein expression at 17 days a.c.

Expression of sFRP-1, sFRP-2, sFRP-3 and sFRP-4 mRNA during murine lung development (Table 2).

Whole mount ISH data:

Both sFRP-1 and sFRP-2 were found to be expressed early in the embryonic lung, while sFRP-3 was not present at any developmental age. SFRP-1 and sFRP-2 mRNA expression was present at 10 days a.c., persisted through 11 and 12 days a.c., and declined around 13 days a.c. As deduced from the whole mount expression pattern, it was located in the connective tissue around the epithelial cells of the lung buds and primordia. For sFRP-4, the mRNA was found during the same period of embryonic development, but the expression pattern indicated an epithelial localization, notably in the apical side of the cytoplasm.

We examined the expression and protein distribution of several Wnt pathway components during prenatal mouse lung development using whole-mount *in situ* hybridization and immunohistochemistry. Between embryonic days 10.5 and 17.5 (E10.5-E17.5), β-catenin was localized in the cytoplasm, and often also the nucleus, of the undifferentiated primordial epithelium (PE), differentiating alveolar epithelium (AE) (present from E14.5 onward), and adjacent mesenchyme. *Tcf1, Lef1, Tcf3, Tcf4, sFrp1, sFrp2* and *sFrp4* were also expressed in the PE, AE, and adjacent mesenchyme in specific spatio-temporal patterns.

These results have been published in the December issue of Tebar et al., Mechanisms of Development, vol. 109/2, 437-440, 2001 (incorporated herein by reference).

RT-PCR

Expression of sFrp1, sFrp2, sFrp3, sFrp4, Dkk1, Dkk2, Dkk3, Fz1, Fz2, Fz3, Fz4, Fz5, Fz6, Fz7, Fz8, Fz9, β-catenin, Tcf1, Lef1, Tcf3 and Tcf4, differentiation

markers SP-A and SP-C, and control RNAs β-actin and GAPDH, was found in lungs dissected from mice of all ages analyzed, *i.e.*, E12, E13, E14, E15, E16, E17, E18, neonates, 1 week olds, 3 week olds and adults.

Potential differences in expression levels were found for *SP-A*, *SP-C* and *sFrp3*. Expression of different isoforms was found for *Tcf-1* and *Lef-1*.

Example 2. *Activation of alveolar type II cells in a murine lung by influencing a Wnt-pathway*

In the murine lung cultures, the oligonucleotides were found to be delivered to embryonic, neonatal, and/or adult lung cells or to pools of mixed ages within 3 hours following their administration. At that time (day 0), the lung cells were dispersed throughout the wells and did no show any (alveolar or other) pattern formation. On day 1, control cultures of lung cells of single or mixed ages showed no changes or, sometimes, a single greyish/black area (Fig. 1A). However, stimulation with bovine pituitary extract (BPE; containing growth factors such as the keratinocyte growth factor capable of inducing epithelial growth/differentiation) resulted in the development of more greyish/black areas, representing developing airspaces (Fig. 1B). The use of sFRP-3 anti-sense oligonucleotide (Fig. 1C) and Dkk-1 anti-sense oligonucleotide (Fig. 1D) also led to the formation of air spaces. Sham treatment of the lung cells with control oligonucleotides did not influence the culture morphology beyond control level, see Fig. 1A.

The developing airspaces were quantified over time. As mentioned above, only one or even no airspaces were present in the control wells at day 1. This outcome did not change markedly during the culture. In the BPE

treated wells there were on average at least five developing airspaces visible, which number again did not change markedly over time.
In the sFRP-3 and Dkk-1 treated wells, however, on average the number of developing airspaces increased from 6 and 9, respectively, to 11 and 14 already at 6 days *in vitro*.

In other sets of experiments, the Dkk-1 anti-sense oligonucleotide - by inhibiting the Wnt pathway inhibitor Dkk-1 from expression - again led to the formation of additional airspaces. As shown in Figure 2 (6 days in culture), the control mixed organoid lung culture (A) showed a low level of airspace formation, whereas in the BPE stimulated wells (B) the level of airspace formation was much higher. The Dkk-1 stimulated wells (D) also showed many airspaces, although on the average smaller in size than the BPE stimulated wells. The number of these airspaces was higher than that in the control wells. The sFRP-1 anti-sense oligonucleotide (C) on the other hand seemed to inhibit airspace formation.

In conclusion, it is shown that the use of anti-sense oligonucleotides inhibiting some inhibitors of the Wnt pathway influences the development of airspaces in the developing murine lung.

RT-PCR (OLC, mouse)

Expression of sFrp1, sFrp2, sFrp3, sFrp4, Dkk1, Dkk2, Dkk3 and Lef-1, differentiation markers SP-A and SP-C, and control RNAs β-actin and GAPDH was found in organoid lung cultures, cultured for different times and/or under different conditions (with BPE; with anti-sense oligonucleotides for sFrp1, sFrp2, sFrp3, sFrp4, Dkk1, Dkk2 or Dkk3; or combinations thereof).

Example 3. Expression of Wnt signalling pathways components in human lung tissues

RT-PCR (emphysematous lungs from patients; control lungs)

Expression of sFRP1 was observed in one normal lung specimen, while another was negative. Of three specimens of emphysematous lung two were positive for sFRP1 and one was negative. Expression of sFRP2, sFRP3 and sFRP4 was positive in all five samples, while sFRP5 was negative in all five samples.

Dkk-1 was found not to be expressed in two samples of normal lung tissue and 3 samples of emphysematous lung tissue.

Dkk-2 and Dkk-3 were found to be expressed in all samples of normal and emphysematous lung tissue. Dkk-4 expression was observed in one of two normal lung specimens (the same specimen that was positive for sFRP1) while the other normal sample was negative.

All three emphysematous lung samples were found negative for Dkk-4 expression.

Table 1. Whole mount *in situ* hybridization data for Lef/Tcfs mRNA expression through alveolar development in the mouse embryo

	10*		11		12		13		14		15		16		17		18		19		Adult
	Epi	Mes	Epi	Mes	Epi	Mes	Epi	Mes	Epi	Mes	Epi	Mes	Epi	Mes	Epi	Mes	Epi	Mes	Epi	Mes	
Lef-1	-	+/-	-	+/+	-	++/+	-	++/+	-	++/+	-	++/+	-	+/-	-	-	-	-	-	-	-
Tcf-1	+/-		+/+		+/+		++/+		++/+		++/+		+		+/-		+/-		-		+/-
Tcf-3	+/+	-	++	-	+++	-	+++	-	++/+	-	++/+	-	++/+	-	+/-	-	-	-	-	-	-
Tcf-4	++	-	++	-	++/+	-	+/+	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-

*Days after conception.

Epi: distal epithelium

Mes: surrounding mesenchyme

Table 2. Whole mount *in situ* hybridization data for sFRPs mRNA expression through alveolar development in the mouse embryo

	10*		11		12		13		14		15		16		17		18		19		Adult
	Epi	Mes	Epi	Mes	Epi	Mes	Epi	Mes	Epi	Mes	Epi	Mes	Epi	Mes	Epi	Mes	Epi	Mes	Epi	Mes	
sFRP1	-	++	-	+++	-	++	-	+/+	-	+	-	-	-	-	-	-	-	-	-	-	+/-
sFRP2	-	++	-	+++	-	++	-	+/+	-	+	-	-	-	-	-	-	-	-	-	-	+/-
sFRP3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
sFRP4	++	-	+++	-	++	-	++	-	+/+	-	+	-	-	-	-	-	-	-	-	-	+/+

*Days after conception.

Epi: distal epithelium

Mes: surrounding mesenchyme

Brief description of the drawings

Figure 1. Overview of murine organoid lung culture wells containing lung cells of various gestation times and neonatal and adult lung cells after 1 day in culture. A, control; B, BPE treated; C, sFRP-3 oligo treated; D, Dkk-1 oligo treated.

Figure 2. Overview of murine organoid lung culture wells containing lung cells ofvarious gestation times and neonatal and adult lung cells after 6 days in culture. A, control; B, BPE treated; C, sFRP-1 oligo treated; D, Dkk-1 oligo treated.

References

Augustine K, Liu ET and Sadler TW. 1993. Antisense attenuation of Wnt-1 and Wnt-3a expression in whole embryo culture reveals roles for these genes in craniofacial, spinal cord, and cardiac morphogenesis. Dev Genet, 14:500-20.

Augustine KA, Liu ET, Sadler TW. 1995. Interactions of Wnt-1 and Wnt-3a are essential for neural tube patterning. Teratology 2: 107-19.

Barker N and Clevers H. 2000. Catenins, Wnt signaling and cancer. Bioessays, 22:961-965.

Behrens J, von Kries JP, Kuhl M, Bruhn L, Wedlich D, Grosschedl R and Birchmeier W. 1996. Functional interaction of beta-catenin with the transcription factor LEF-1. Nature, 382:638-42.

Bhanot P, Brink M, Samos CH, Hsieh JC, Wang Y, Macke JP, Andrew D, Nathans J and Nusse R. 1996. A new member of the frizzled family from Drosophila functions as a Wingless receptor. Nature, 382:225-30.

Bienz M, Clevers H. 2000 Linking colorectal cancer to Wnt signaling. Cell.103:311-20.

Brandsma AE, Ten Have-Opbroek AAW, Vulto IM, Molenaar JC, Tibboel, D. 1994. Alveolar epithelial composition and architecture of the late fetal pulmonary acinus. An immunocytochemical and morphometric study in a rat model of pulmonary hypoplasia and congenital diaphragmatic hernia. Exp Lung Res 20:491-515.

Brisken C, Heineman A, Chavarria T, Elenbaas B, Tan J, Dey SK, McMahon JA,McMahon AP, Weinberg RA. 2000 Essential function of Wnt-4 in mammary gland development downstream of progesterone signaling.Genes Dev. 14:650-4.

Brown JD, Hallagan SE, McGrew LL, Miller JR and Moo RT. 2000. The maternal Xenopus beta-catenin signaling pathway, activated by frizzled homologs, induces goosecoid in a cell non-autonomous manner. Dev Growth Differ, 42:347-57.

Buhler TA, Dale TC, Kieback C, Humphreys RC, Rosen JM. 1993 Localization and quantification of Wnt-2 gene expression in mouse mammary development. Dev Biol.155:87-96.

Burge PS, Brit Med J 2000; 320:1297-1303

Calvo R, West J, Franklin W, Erickson P, Bemis L, Li E, Helfrich B, Bunn P,Roche J, Brambilla E, Rosell R, Gemmill RM, Drabkin HA. 2000 Altered HOX and WNT7A expression in human lung cancer. Proc Natl Acad Sci U S A. 97:12776-81

Cho EA, Dressler GR. 1998 TCF-4 binds beta-catenin and is expressed in distinct regions of the embryonic brain and limbs. Mech Dev. 77:9-18

Christiansen JH, Dennis CL, Wicking CA, Monkley SJ, Wilkinson DG and Wainwright BJ. 1995. Murine Wnt-11 and Wnt-12 have temporally and spatially restricted expression patterns during embryonic development. Mech Dev, 51:341-50.

Dagle JM, Littig JL, Sutherland LB, Weeks DL. 2000. Targeted elimination of zygotic messages in Xenopus

laevis embryos by modified oligonucleotides possessing terminal cationic linkages. Nucl Acids Res 28: 2153-2157.

Galceran J, Farinas I, Depew MJ, Clevers H, Grosschedl R.1999 Wnt3a-/--like phenotype and limb deficiency in Lef1(-/-)Tcf1(-/-) mice. Genes Dev. 13:709-17

Gavin BJ, McMahon JA, McMahon AP. 1990 Expression of multiple novel Wnt-1/int-1-related genes during fetal and adult mouse development. Genes Dev. 12B:2319-32.

Glinka A, Wu W, Delius H, Monaghan AP, Blumenstock C, Niehrs C. 1998. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. Nature. 391: 357-362.

Goedbloed JF. 1976. Embryonic and postnatal growth of rat and mouse. IV. Prenatal growth of organs and tissues: age determination, and general growth pattern. Acta Anat (Basel), 95:8-33.

Ten Have-Opbroek AAW. Immunological study of lung development in the mouse embryo. I. Appearance of a lung-specific antigen, localized in the great alveolar cell. Dev Biol 46:390-403, 1975.

Ten Have-Opbroek AAW. Immunological study of lung development in the mouse embryo. II. First appearance of the great alveolar cell, as shown by immunofluorescence microscopy. Dev Biol 69:408-423, 1979.

Ten Have-Opbroek AAW. The development of the lung in mammals: An analysis of concept and findings. Am J Anat 162:201-219, 1981.

Ten Have-Opbroek AAW, Dubbeldam JA, Otto-Verberne CJM. Ultrastructural features of type II alveolar epithelial cells in early embryonic mouse lung. Anat Rec 221:846-853, 1988.

Ten Have-Opbroek AAW, Otto-Verberne CJM, Dubbeldam JA. Ultrastructural characteristics of inclusion bodies of type II cells in late embryonic mouse lung. Anat Embryol 181:317-323, 1990.

Ten Have-Opbroek AAW, Hammond WG, Benfield JR. Bronchiolo-alveolar regions in adenocarcinoma arising from canine segmental bronchus. Cancer Letters 55:177-182, 1990.

Ten Have-Opbroek AAW. *Invited review.* Lung development in the mouse embryo. Exp Lung Res 17:111-130, 1991.

Ten Have-Opbroek AAW, Plopper CG. Morphogenetic and functional activity of type II cells in early fetal Rhesus monkey lungs. A comparison between primates and rodents. Anat Rec 234:93-104, 1992.

Ten Have-Opbroek AAW, Hammond WG, Benfield JR, Teplitz RL, Dijkman JH. Expression of alveolar type II cell markers in acinar adenocarcinomas and adenoid-cystic carcinomas arising from segmental bronchi. A study in a heterotopic bronchogenic carcinoma model in dogs. Am J Pathol 142:1251-1264, 1993.

Ten Have-Opbroek AAW, Benfield JR, Hammond WG, Teplitz RL, Dijkman JH. *Invited review.* In favour of an oncofoetal concept of bronchogenic carcinoma development. Histol Histopath 9:375-384, 1994.

Ten Have-Opbroek AAW, Benfield JR, Hammond WG, Dijkman JH. Alveolar stem cells in canine bronchial carcinogenesis. Cancer Lett 101:211-217, 1996.

Ten Have-Opbroek AAW, Benfield JR, Van Krieken JHJH, Dijkman JH. The alveolar type II cell is a pluripotential stem cell in the genesis of human adenocarcinomas and squamous cell carcinomas. Histol Histopathol 12:319-336, 1997.

Ten Have-Opbroek AAW, Shi X-B, Gumerlock PH. 3-Methylcholanthrene triggers the differentiation of alveolar tumor cells from canine bronchial basal cells and an altered p53 gene promotes their clonal expansion. Carcinogenesis 21:1477-1484, 2000.

Heasman J, Kofron M, Wylie C. 2000. Beta-catenin signaling activity dissected in the early Xenopus embryo: a novel antisense approach. Dev Biol 222:124-134.

Heemskerk-Gerritsen BAM, Dijkman JH, Ten Have-Opbroek AAW. 1996. Stereological methods: A new approach in the assessment of pulmonary emphysema. Microsc Res Techn 34:556-562.

Hinck L, Nelson WJ and Papkoff J. 1994. Wnt-1 modulates cell-cell adhesion in mammalian cells by stabilizing beta-catenin binding to the cell adhesion protein cadherin. J Cell Biol, 124:729-41.

Huber O, Korn R, McLaughlin J, Ohsugi M, Herrmann BG, Kemler R.1996 Nuclear localization of beta-catenin by interaction with transcription factor LEF-1.Mech Dev.59:3-10

Huelsken J, Vogel R, Brinkmann V, Erdmann B, Birchmeier C and Birchmeier W. 2000. Requirement for beta-catenin in anterior-posterior axis formation in mice. J Cell Biol, 148:567-78.

Hundertmark S et al. 1999. Effect of dexamethasone, triiodothyronine and dimethyl-isopropyl thyronine on lung maturation of the fetal rat lung. J Perinat Med 27:309-315.

Imai K, D'Armiento J. Expression of Wnt10b and sFRP1 in embryonic mouse lung. Am Rev Resp Crit Care Med 159:A817, 1999

Katoh M, Hirai M, Sugimura T, Terada M. 1996 Cloning, expression and chromosomal localization of Wnt-13, a novel member of the Wnt gene family. Oncogene. 13:873-6

Kispert A, Vainio S and McMahon AP. 1998. Wnt-4 is a mesenchymal signal for epithelial transformation of metanephric mesenchyme in the developing kidney. Development, 125:4225-34.

Korinek V, Barker N, Willert K, Molenaar M, Roose J, Wagenaar G, Markman M, Lamers W, Destree O and Clevers H. 1998. Two members of the Tcf family implicated in Wnt/beta-catenin signaling during embryogenesis in the mouse. Mol Cell Biol, 18:1248-56.

Lako M, Strachan T, Bullen P, Wilson DI, Robson SC and Lindsay S.1998. Isolation, characterisation and embryonic expression of WNT11, a gene which maps to 11q13.5 and has possible roles in the development of skeleton, kidney and lung. Gene, 219:101-10.

Lee SM, Tole S, Grove E and McMahon AP. 2000. A local Wnt-3a signal is required for development of the mammalian hippocampus. Development, 127:457-67.

Leimeister C, Bach A, Gessler M. 1998 Developmental expression patterns of mouse sFRP genes encoding members of the secreted frizzled related protein family. Mech Dev. 75):29-42

Levay-Young BK and Navre M. 1992. Growth and developmental regulation of wnt-2 (irp) gene in mesenchymal cells of fetal lung. Am J Physiol, 262(6 Pt 1):L672-83.

Leyns L, Bouwmeester T, Kim SH, Piccolo S and De Robertis EM. 1997. Frzb-1 is a secreted antagonist of Wnt signaling expressed in the Spemann organizer. Cell, 88:747-56.

Liu P, Wakamiya M, Shea MJ, Albrecht U, Behringer RR, Bradley A. 1999 Requirement for Wnt3 in vertebrate axis formation Nat Genet. 22:361-5

McMahon AP, Bradley A. 1990 The Wnt-1 (int-1) proto-oncogene is required for development of a large region of the mouse brain.Cell. 62:1073-85

McMahon AP, Gavin BJ, Parr B, Bradley A and McMahon JA.1992. The Wnt family of cell signalling molecules in postimplantation development of the mouse. Ciba Found Symp, 165:199-212; discussion 212-8.

Miller JR, Moon RT. 1996 Signal transduction through beta-catenin and specification of cell fate during embryogenesis.Genes Dev. 10:2527-39

Molenaar M, van de Wetering M, Oosterwegel M, Peterson-Maduro J, Godsave S, Korinek V, Roose J, Destree O, Clevers H.1996 XTcf-3 transcription factor mediates beta-catenin-induced axis formation in Xenopus embryos. Cell. 86:391-9

Monkley SJ, Delaney SJ, Pennisi DJ, Christiansen JH, Wainwright BJ.1996 Targeted disruption of the Wnt2 gene results in placentation defects. Development. 122:3343-53

Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, Kinzler KW. 1997 Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science. 275:1787-90.

Nieto MA, Patel K and Wilkinson DG. 1996. In situ hybridization analysis of chick embryos in whole mount and tissue sections. Methods Cell Biol, 51:219-35.

Nusse R and Varmus HE. 1992. Wnt genes. Cell, 69:1073-87.

Oosterwegel M, van de Wetering M, Timmerman J, Kruisbeek A, Destree O, Meijlink F, Clevers H. Differential expression of the HMG box factors TCF-1 and LEF-1 during murine embryogenesis.Development. 118:439-48

Otto-Verberne CJM, Ten Have-Opbroek AAW. Development of the pulmonary acinus in fetal rat lung: a study based on an antiserum recognizing surfactant-associated proteins. Anat Embryol 175:365-373, 1987.

Otto-Verberne CJM, Ten Have-Opbroek AAW, Balkema JJ, Franken C. Detection of the type II cell or its precursor before week 20 of human gestation, using antibodies against surfactant-associated proteins. Anat Embryol 178:29-39, 1988.

Otto-Verberne CJM, Ten Have-Opbroek AAW, De Vries ECP. Expression of the major surfactant-associated protein, SP-A, in type II cells of human lung before 20 weeks of gestation. Eur J Cell Biol 53:13-19, 1990.

Otto-Verberne CJM, Ten Have-Opbroek AAW, Willems LNA, Franken C, Kramps JA, Dijkman JH. 1991. Lack of type II cells and emphysema in human lungs. Eur Respir J 4:316-323.

Parr BA, Shea MJ, Vassileva G, McMahon AP. Mouse Wnt genes exhibit discrete domains of expression in the early embryonic CNS and limb buds.Development. 119:247-61

Pauwels RA et al., New Engl J Med 1999: 340:1948-1953

Polakis P. 2000. Wnt signaling and cancer. Genes Dev, 14:1837-51.

Porter JD and Baker RS.1997. Absence of oculomotor and trochlear motoneurons leads to altered extraocular muscle development in the Wnt-1 null mutant mouse. Brain Res Dev Brain Res, 100:121-6.

Roelink H, Nusse R.1991 Expression of two members of the Wnt family during mouse development restricted temporal and spatial patterns in the developing neural tube. Genes Dev. 3:381-8

Rubinfeld B, Albert I, Porfiri E, Munemitsu S and Polakis P. 1997. Loss of beta-catenin regulation by the APC tumor suppressor protein correlates with loss of structure due to common somatic mutations of the gene. Cancer Res, 57:4624-30.

Sarkar L and Sharpe PT. 1999. Expression of Wnt signalling pathway genes during tooth development. Mech Dev, 85:197-200.

Stark K, Vainio S, Vassileva G and McMahon AP.1994. Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. Nature, 372:679-83.

Takada S, Stark KL, Shea MJ, Vassileva G, McMahon JA and McMahon AP. 1994. Wnt-3a regulates somite and tailbud formation in the mouse embryo. Genes Dev, 8:174-89.

Tebar M, Destree O, De Vree WJA, Ten Have-Opbroek AAW. 2001. Expression of Tcf/Lef and sFRP and localization of ß-catenin in the developing mouse lung. Mechanisms of Development. 109/2: 437-440.

Wang J, Shackleford GM.Murine Wnt10a and Wnt10b 1996 cloning and expression in developing limbs, face and skin of embryos and in adults. Oncogene. 13:1537-44

Wang S, Krinks M, Lin K, Luyten FP and Moos M Jr. 1997. Frzb, a secreted protein expressed in the Spemann organizer, binds and inhibits Wnt-8. Cell, 88:757-66.

Wilkinson DG. 1995. RNA detection using non-radioactive in situ hybridization. Curr Opin Biotechnol, 6:20-23.

Wilkinson DG and Nieto MA. 1993. Detection of messenger RNA by in situ hybridization to tissue sections and whole mounts. Methods Emzymol, 225:361-373.

Willert K and Nusse R. 1998. Beta-catenin: a key mediator of Wnt signaling. Curr Opin Genet Dev, 8:95-102.

Winn R.A. and West J.B. 2000. Evidence for involvement of the Wnt-pathway in lung cancer. Am.J.Respir.Crit.Care.Med. 161, A670.

Wodarz A and Nusse R. 1998. Mechanisms of Wnt signaling in development. Annu Rev Cell Dev Biol, 14:59-88.

Yamaguchi TP, Bradley A, McMahon AP, Jones S. 1999 A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo.Development 126:1211-23.

Zakin LD, Mazan S, Maury M, Martin N, Guenet JL, Brulet P. 1998 Structure and expression of Wnt13, a novel mouse Wnt2 related gene. Mech Dev. 73:107-16

Zimmermann B. 1987. Lung organoid culture. Differentiation 36: 86-109.

Zimmermann B. 1989. Secretion of lamellar bodies in type II pneumocytes in organoid culture: Effects of colchicine and cytochalasin B. Exp Lung Res 15:31-47.

Claims

1. A composition capable of influencing the proliferation and/or differentiation behavior of an alveolar type II cell and/or an alveolar type II tumor cell from a lung, comprising a nucleic acid capable of binding at least a functional part of a nucleic acid encoding a protein which is involved in a Wnt-pathway in said cell, said binding influencing said Wnt-pathway.
2. A composition capable of influencing the proliferation and/or differentiation behavior of an alveolar type II cell and/or an alveolar type II tumor cell from a lung, comprising a protein capable of binding at least a functional part of a protein which is involved in a Wnt-pathway in said cell, or at least a functional part of a nucleic acid encoding a protein which is involved in a Wnt-pathway in said cell, said binding influencing said Wnt-pathway.
3. A composition according to claim 1 or 2, wherein said cell is located inside a body of a human or animal.
4. A composition according to anyone of claims 1-3, wherein said Wnt-pathway is upregulated.
5. A composition according to claim 4, which is at least in part capable of inhibiting expression of at least one secreted Frizzled-related protein and/or Dickkopf protein.
6. A compound according to claim 5, which at least comprises one antisense strand of at least a functional part of DNA and/or RNA encoding at least part of secreted Frizzled-related protein and/or Dickkopf protein.
7. A compound according to anyone of claims 1-6, which is capable of at least in part counteracting a Wnt-

pathway inhibiting property of at least one secreted Frizzled-related protein and/or Dickkopf protein.

8. A compound according to anyone of claims 1-7, which is capable of binding to at least one secreted Frizzled-related protein and/or Dickkopf protein.
9. A compound according to anyone of claims 1-8, which comprises an antibody comprising a binding specificity against a secreted Frizzled-related protein and/or Dickkopf protein, or a functional part, derivative and/or analogue of said antibody.
10. A compound according to anyone of claims 5-9, wherein said Frizzled-related protein is sFRP-1, sFRP-2, sFRP-3, and/or sFRP-4.
11. A compound according to anyone of claims 5-10, wherein said Dickkopf protein is Dkk1, Dkk2 and/or Dkk3.
12. A compound according to anyone of claims 1-11, which is capable of activating expression of at least one transcription factor of the TCF/LEF family.
13. A compound according to anyone of claims 1-12, which at least comprises one nucleic acid encoding a transcription factor of the TCF/LEF family or a functional part, derivative and/or analogue thereof.
14. A compound according to claim 12 or 13, wherein said transcription factor of the TCF/LEF family is TCF-1, TCF-3, TCF-4 and/or LEF-1.
15. A compound according to anyone of claims 1-14, which is capable of inducing the formation of an alveolar bud.
16. A compound according to anyone of claims 1-15, which is capable of inducing synthesis and/or secretion of surfactant by a lung cell.
17. An isolated cell, comprising a compound according to anyone of claims 1-16.
18. A vector comprising a nucleic acid capable of binding at least a functional part of a nucleic acid encoding

a protein which is involved in a Wnt-pathway in a cell, said binding influencing said Wnt-pathway.

19. A vector comprising a nucleic acid encoding a protein capable of binding at least a functional part of a protein which is involved in a Wnt-pathway in a cell, or at least a functional part of a nucleic acid encoding a protein which is involved in a Wnt-pathway in a cell, said binding influencing said Wnt-pathway.
20. Use of a compound according to anyone of claims 1-16 for the preparation of a medicament.
21. Use of a compound according to anyone of claims 1-16 for the preparation of a medicament for emphysema.
22. Use of a compound according to anyone of claims 1-16 for the preparation of a medicament for Respiratory Distress Syndrome.
23. Use of a compound according to anyone of claims 1-16 for the preparation of a medicament for lung cancer.
24. A method for inducing the formation of an alveolar bud, comprising administering a compound according to anyone of claims 1-16 to an alveolar type II cell.
25. A method for inducing synthesis and/or secretion of surfactant by a cell, comprising administering a compound according to anyone of claims 1-16 to said cell.
26. A method according to claim 25, wherein said cell is an alveolar type II cell.
27. A method for, at least in part, treatment of emphysema, comprising administering a compound according to anyone of claims 1-16 to an individual.
28. A method for, at least in part, treatment of Respiratory Distress Syndrome, comprising administering a compound according to anyone of claims 1-16 to an individual.
29. A method for, at least in part, treatment of lung cancer, comprising administering a compound according to anyone of claims 1-16 to an individual.

Figure 1

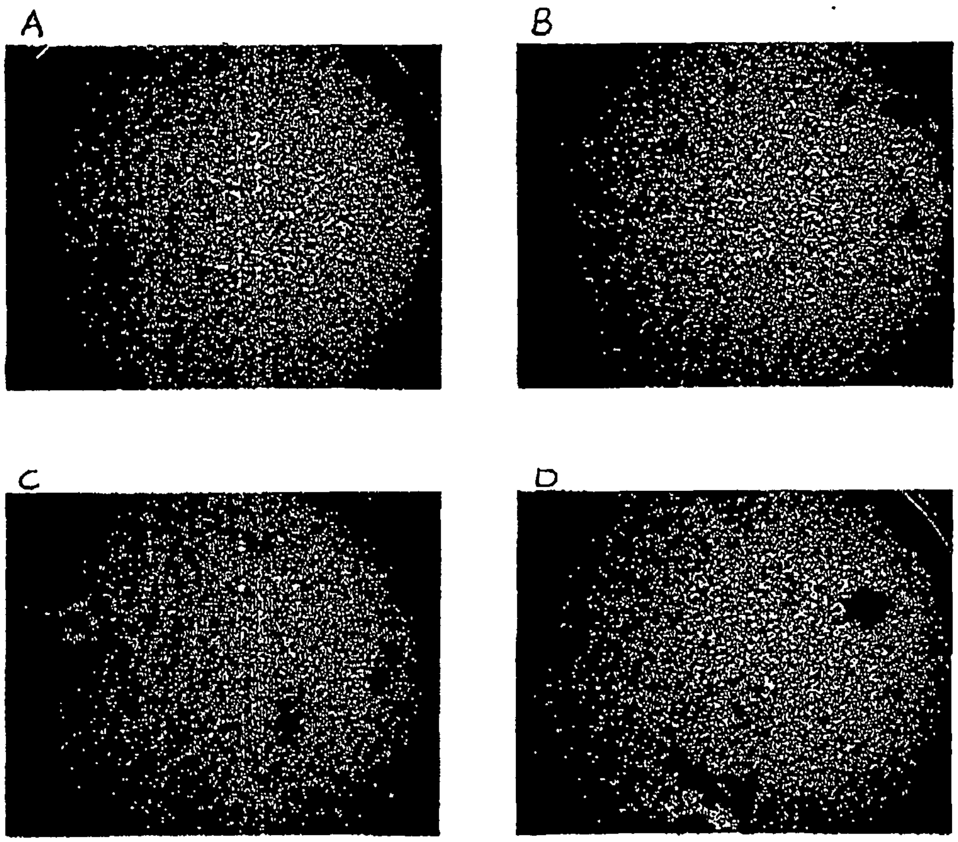
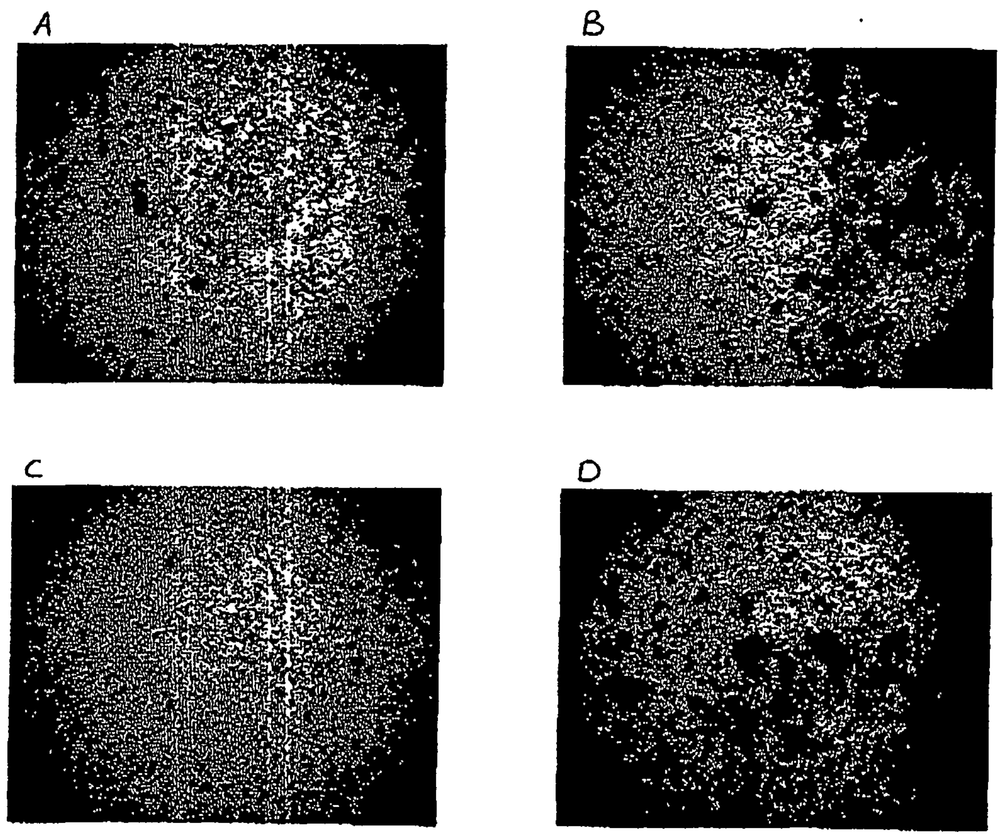


Figure 2



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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/055109 A3

(54) Title: GENERATION AND/OR REDUCTION OF NEW LUNG TISSUE IN AN AFFECTED LUNG, BY MODULATION OF THE WNT-PATHWAY

(57) Abstract: The present invention provides a means to influence the formation and/or reduction of new lung cells, by influencing a Wnt-pathway in an alveolar type II cell and/or alveolar type II tumor cell from said lung. Therefore, the invention provides a composition comprising a nucleic acid capable of binding at least a functional part of a nucleic acid encoding a protein which is involved in a Wnt-pathway in said cell, said binding influencing said Wnt-pathway. A composition of the invention may also comprise a protein capable of binding at least a functional part of a protein which is involved in a Wnt-pathway in said cell, or at least a functional part of a nucleic acid encoding a protein which is involved in a Wnt-pathway in said cell, said binding influencing said Wnt-pathway. A composition of the invention is suitable for the preparation of a medicament against emphysema, Respiratory Distress Syndrome and/or lung cancer.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 02/00025

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K48/00 A61K38/02 C07K14/705 C07K16/18 C12N5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DENNIS S. ET AL: "A secreted Frizzled related protein, FrzA, selectively associates with Wnt-1 protein and regulates Wnt-1 signaling." J. CELL. SCI., vol. 112, 1999, pages 3815-3820, XP002174506 the whole document –––	2,17,18
X	WO 99 22000 A (DEUTSCHES KREBSFORSCH ;GLINKA ANDREI (DE); NIEHRS CHRISTOF (DE)) 6 May 1999 (1999-05-06) the whole document –––	1,2,4-9, 17-20

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 March 2002	05.06.02

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Armandola, E

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	POLANEC J ET AL: "EFFECT OF WNT-1 ANTISENSE RNA ON THE OUTGROWTH OF A MAMMARY ADENOCARCINOMA CELL LINE EXPRESSING THAT ONCOGENE" JCP. CLINICAL MOLECULAR PATHOLOGY, LONDON, GB, vol. 49, no. 3, June 1996 (1996-06), pages 166-169, XP000929749 ISSN: 1355-2910 the whole document	1,8,10, 17-20
Y		2-7,9, 15,16, 21-29
X	DUMONTELLE J ET AL: "Inhibition of expression of the endogenous mammary oncogene Wnt-1 by antisense oligodeoxynucleotides" PROCEEDINGS OF THE ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, NEW YORK, NY, US, vol. 36, March 1995 (1995-03), page 515 XP002147584 ISSN: 0197-016X the whole document	1,17-20
Y		2-10,15, 16,21-29
X	GLINKA ET AL: "Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 391, no. 6665, 22 January 1998 (1998-01-22), pages 357-362, XP002096088 ISSN: 0028-0836 page 360, right-hand column -page 361, left-hand column	2,4,8,9, 17
X	WO 98 13493 A (UMANSKY SAMUIL ;MELKONYAN HOVSEP (RU); LXR BIOTECHNOLOGY INC (US)) 2 April 1998 (1998-04-02) the whole document	1-10, 15-20
X	WO 00 38709 A (CHIRON CORP ;XU LICEN (US); HARRISON STEPHEN D (US); WILLIAMS LEWI) 6 July 2000 (2000-07-06) the whole document	1-4, 15-20, 23,29
Y		5-10,21, 22,24-26

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 52047 A (MILLENNIUM PHARM INC) 8 September 2000 (2000-09-08) page 3 -page 10 page 43 -page 45 page 47 -page 50 page 107, line 22 - line 31	1-9, 15-23, 27-29
X	WO 98 54325 A (US HEALTH) 3 December 1998 (1998-12-03) page 12, line 15 -page 16, line 15 page 20, line 5 -page 21, line 17	1-10, 15-20
Y		21-29
P,X	WO 01 19855 A (AMERICAN HOME PROD ;BODINE PETER V N (US)) 22 March 2001 (2001-03-22) the whole document	1-10, 15-20
P,X	WO 01 44279 A (CHIRON CORP) 21 June 2001 (2001-06-21) page 6 page 24 -page 26 page 34 -page 38	1-4, 15-20
Y	CALVO, R. ET AL.: "Altered HOX and WNT7A expression in human lung cancer" PROC. NATL. ACAD. SCI. USA, vol. 97, no. 23, 7 November 2000 (2000-11-07), pages 12776-12781, XP002174507 the whole document	2-10,15, 16,21-29
A	BARKER N., CLEVERS H.: "Catenins, Wnt signaling and cancer" BIOESSAYS, vol. 22, November 2000 (2000-11), pages 961-965, XP002174508	
A	BIENZ M., CLEVERS H.: "Linking colorectal cancer to Wnt signaling" CELL, vol. 103, 13 October 2000 (2000-10-13), pages 311-320, XP002174510	
A	GILBERT KA AND RANNELS DE: "From Limbs to Lungs: a Newt Perspective on Compensatory Lung Growth" NEWS PHYSIOL. SCI., vol. 14, December 1999 (1999-12), pages 260-267, XP001013538	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL 02/00025

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: –
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 20-29 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 1-10, 15-29 (all in part)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-9 (partially), 10, 15-29 (partially)

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-10, 15-29 (all in part)

Present claims 1-10 and 15-29 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to antisense molecules and antibodies targeted at sFRPs.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-9 (partially), 10, 15-29 (partially)

   A composition capable of influencing the Wnt pathway comprising a compound, the compound being nucleic acids and/or proteins interacting with sFRPs. Isolated cells and vectors comprising them,uses of the composition for making medicaments and methods of treatment of lung diseases utilizing the composition.

2. Claims: 1-9 (partially), 11, 15-29 (partially)

   As 1., the compound being nucleic acids and/or proteins interacting with Dkk proteins.

3. Claims: 1-4 (partially), 12-14, 15-29 (partially)

   As 1. where the compound activates expression of transcription factors of the TCF/LEF family and/or is a nucleic acid encoding a member of the family.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 02/00025

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9922000	A	06-05-1999	DE 19747418	C	15-07-1999
			EP 1027440	A	16-08-2000
			JP 2001520886	T	06-11-2001
WO 9813493	A	02-04-1998	AU 737323	B	16-08-2001
			AU 4651397	A	17-04-1998
			EP 0932678	A	04-08-1999
WO 0038709	A	06-07-2000	AU 2396000	A	31-07-2000
WO 0052047	A	08-09-2000	AU 3510200	A	21-09-2000
			EP 1161445	A	12-12-2001
WO 9854325	A	03-12-1998	AU 7704498	A	30-12-1998
WO 0119855	A	22-03-2001	AU 7131200	A	17-04-2001
			BR 0014183	A	14-05-2002
WO 0144279	A	21-06-2001	AU 2061201	A	25-06-2001